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Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms

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Abstract

Although numerous studies indicate that increasing atmospheric CO_2 or temperature stimulate soil CO_2 efflux, few data are available on the responses of three major components of soil respiration [i.e. rhizosphere respiration (root and root exudates), litter decomposition, and oxidation of soil organic matter] to different CO_2 and temperature conditions. In this study, we applied a dual stable isotope approach to investigate the impact of elevated CO_2 and elevated temperature on these components of soil CO_2 efflux in Douglas-fir terracosms. We measured both soil CO_2 efflux rates and the ^{13}C and ^{18}O isotopic compositions of soil CO_2 efflux in 12 sun-lit and environmentally controlled terracosms with 4-year-old Douglas fir seedlings and reconstructed forest soils under two CO_2 concentrations (ambient and 200 ppmv above ambient) and two air temperature regimes (ambient and 4 °C above ambient). The stable isotope data were used to estimate the relative contributions of different components to the overall soil CO_2 efflux. In most cases, litter decomposition was the dominant component of soil CO_2 efflux in this system, followed by rhizosphere respiration and soil organic matter oxidation. Both elevated atmospheric CO_2 concentration and elevated temperature stimulated rhizosphere respiration and litter decomposition. The oxidation of soil organic matter was stimulated only by increasing temperature. Release of newly fixed carbon as root respiration was the most responsive to elevated CO_2, while soil organic matter decomposition was most responsive to increasing temperature. Although some assumptions associated with this new method need to be further validated, application of this dual-isotope approach can provide new insights into the responses of soil carbon dynamics in forest ecosystems to future climate changes.

Keywords: elevated CO_2, forest ecosystem, global warming, soil respiration, stable isotopes

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Introduction

Soils are the major reservoir of carbon in terrestrial ecosystems, containing more than two-thirds of total carbon in the terrestrial part of the biosphere. A major unknown in the response to anticipated climate changes is the extent to which forest ecosystems will become net sinks or sources of CO_2. This uncertainty is in part driven by our lack of knowledge on how much root respiration will increase under elevated atmospheric CO_2 and in part on how elevated temperatures might accelerate the turnover of soil organic carbon. Understanding soil carbon dynamics under elevated atmospheric CO_2 and temperature is thus critical for predicting future regional and global carbon budgets (Schimel 1995).

Previous studies have suggested that increasing atmospheric [CO_2] and temperature can stimulate soil CO_2 efflux (e.g. van Veen *et al.* 1991; Körner & Arnone 1992; Peterjohn *et al.* 1993, 1994; Johnson *et al.* 1994; Nakayama *et al.* 1994; Pajari 1995; Vose *et al.* 1995; Hungate *et al.* 1997). However, there is relatively little information on which components of the soil CO_2 efflux are most sensitive to changes in atmospheric CO_2 (see review by Paterson

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et al. 1997) or temperature. The components of soil CO_2 efflux can be partitioned into root respiration, microbial decomposition of soil organic matter, and microbial decomposition of surface-layer litter. Traditionally, radioactive materials have been used to partition components of the CO_2 efflux, including continuous ^{14}C-labelling (e.g. Whipps & Lynch 1983; Merckx *et al.* 1985) and ^{14}C pulse-labelling (e.g. Cheng *et al.* 1996). These methods have environmental health restrictions and thus are limited to short-term experiments, lasting perhaps several months at most (Cheng *et al.* 1996). In ecosystems where the photosynthetic pathway of the current vegetation (C3 or C4) is distinct from the vegetation responsible for the bulk of the soil organic matter accumulation, stable isotopes of carbon have been used to partition soil CO_2 efflux into old vs. recently formed soil carbon components (e.g. Schonwitz *et al.* 1986; Wedin *et al.* 1995; Cheng 1996). However, such transition ecosystems are limited in distribution. A recent variation on this approach has been to add a C4 sugar substrate to the C3-dominated soil to quantify the microbial respiration component of the CO_2 efflux (Högberg & Ekblad 1996). Leavitt *et al.* (1996) and Nitschelm *et al.* (1997) demonstrated that the CO_2 source in Free Air CO_2 Enrichment (FACE) experiments was usually sufficiently different from atmospheric CO_2 that this could also be used as a label to partition CO_2 efflux from old vs. recently formed soil carbon components. However, the amount of carbon added to the soil carbon pool in a 1-or 2-year period was too small, restricting the utility of this approach.

We show here that by measuring changes in the isotopic composition of the soil CO_2 efflux instead of changes in the soil carbon pool in elevated CO_2 studies, larger and more reliable estimates of the soil carbon dynamics may be obtained. Our approach is to use a combination of analyses of the stable isotope ratios of carbon and oxygen in CO_2 efflux with soil water at different depths to partition soil CO_2 efflux into three distinct components: rhizosphere respiration (including root respiration and microbial respiration resulting from consumption of root exudates), microbial decomposition of surface litter, and microbial decomposition of soil organic matter (SOM). This approach overcomes potential concerns about the unnatural mixing of soils and plants, which would otherwise be a significant limitation for the study of natural responses by ecosystems. We apply this approach to analyse the soil CO_2 efflux responses in Douglas fir terracosms with tree seedlings growing under elevated CO_2 and air temperature treatments.

Materials and methods

Elevated CO_2 and temperature treatments

At the U.S. Environmental Protection Agency's Terrestrial Ecophysiology Research Area (TERA), a study of ecosystem response to elevated CO_2 and temperature was initiated in July 1993, using 12 sun-lit controlled-environment terracosm (see Tingey *et al.* 1996 for a detailed description of this facility). Each terracosm had a canopy volume of 3.18 m^3 (2 m wide, 1.5 m tall at the back, 1.2 m tall at the front, 1 m front-to-back) and a soil lysimeter volume of 2.0 m^3 (2 m wide, 1 m front-to-back, 1 m deep). The treatments imposed were: (i) ambient CO_2 and ambient temperature, (ii) elevated CO_2 (ambient plus 200 ppmv) and ambient temperature, (iii) ambient CO_2 and elevated temperature (ambient plus 40 °C), and (iv) elevated CO_2 and elevated temperature (Fig. 1). The soil was a fine loam to loam texture with a medium granular structure and was classified as a coarse-loamy, mixed, frigid Typic Hapludand. At the beginning of the experiment, soil carbon content averaged at 2.38% for A Horizon and 2.07% for B Horizon. Litter from an old-growth Douglas-fir forest was collected and added to the terracosm when the trees were planted (Tingey *et al.* 1996). Seedlings in each terracosm were irrigated with local well water having the same isotopic compositions to maintain similar soil moisture. However, the litter layer tended to be drier in the teracosms than in natural Douglas fir forest due to the control capability. As a result, there were few roots growing in this layer.

The terracosms were maintained as closed systems

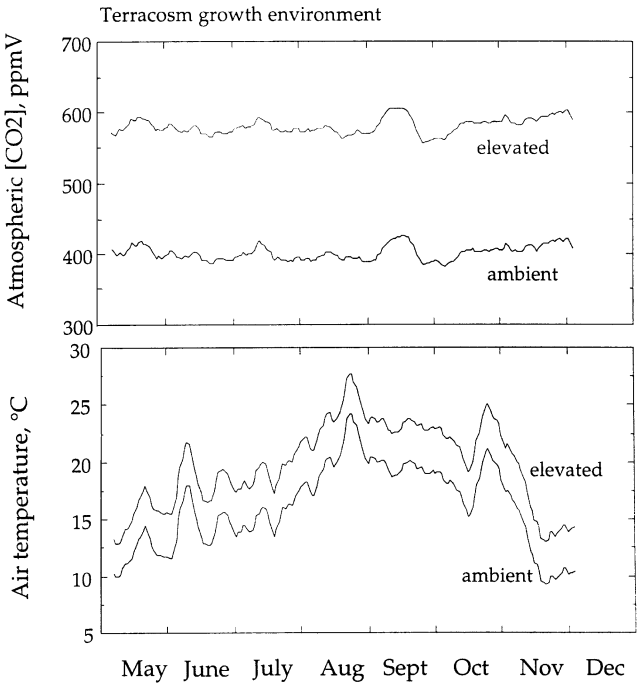

Fig. 1 (a) Seasonal change in CO_2 concentration in the ambient (n = 6) and elevated CO_2 treatment terracosms (n = 6) and (b) seasonal change in air temperature in the ambient (n = 6) and elevated temperature treatment terracosms (n = 6) with Douglas-fir seedlings and reconstructed forest soils.

Table 1 Carbon isotope ratios of the CO_2 from the source tank and of the atmosphere outside but near the terracosms. Values are the mean of 4 replicates and standard error of the mean

	$\delta^{13}C$ (‰)	
Sampling Month	Tank CO_2	Atmospheric CO_2
April 1994	– 35.64 ± 0.07	– 8.83 ± 0.09
June 1994	– 35.84 ± 0.04	– 8.54 ± 0.36
August 1994	– 35.77 ± 0.12	– 8.24 ± 0.27
October 1994	– 35.81 ± 0.23	– 8.63 ± 0.12
Mean	– 35.77 ± 0.05	– 8.55 ± 0.13

most of time during the study (opened only whenever needed for maintenance, sampling or physiological measurements). Commercial tank CO_2 and carbon dioxide scrubbers were used to maintain the desired CO_2 concentrations both in the ambient and elevated CO_2 treatment terracosms (Tingey *et al.* 1996). During day time, tank CO_2 was added to compensate the photosynthetic uptake and to maintain a specific CO_2 concentration, while at nights the excessive CO_2 from soil and plant respiration was removed using Soda Lime scrubbers. Atmospheric CO_2 concentration in each terracosm was monitored continuously using a LI-6262 CO_2/H_2O analyser (LI-COR Inc., Lincoln, Nebraska, USA) which was calibrated regularly with CO_2 standards. Efforts were made to use tank CO_2 with relatively low and constant ^{13}C content throughout the entire study (Table 1). As a consequence, new photosynthate produced during the experimental period and allocated to the leaves and roots had a different ^{13}C content than previously grown material. Repeated measurement of the outside atmospheric and tank CO_2 confirmed that source atmospheric CO_2 values remained constant throughout the growing season (Table 1). The mean carbon isotope ratio of the tank CO_2 was – 35.77 ± 0.09‰, compared with – 8.55 ± 0.13‰ for the atmospheric CO_2 at the TERA area. The temperature in each terracosm was controlled at specific levels through the use of heat exchangers.

Soil CO_2 efflux measurements

During four sampling periods in 1994 (16–22 April, 20–22 June, 17–21 August and 18–20 October), soil CO_2 efflux in each terracosm was measured at two locations using a LI-6000–09 soil respiration chamber (LI-COR Inc., Lincoln, NB, USA) and PVC collars (10 cm diameter × 7 cm height). For each sampling location, a PVC collar was installed in March 1994 to a depth of 5 cm in the soil and left undisturbed throughout the entire study. The volume of free space above the litter surface contained within each soil collar was measured prior to each soil CO_2 efflux measurement. During each measurement, change in the CO_2 concentration in the closed chamber over 1–2 min was measured using the CO_2 analyser of a LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) and used to calculate soil CO_2 efflux rate. In addition, the soil temperature at 5-cm depth was measured with a thermocouple sensor attached to the LI-6000–09 soil respiration chamber.

Isotope sampling and analyses of soil CO_2 efflux and organic matter

After a soil CO_2 efflux rate had been measured, CO_2 emitted from the soil was collected by inserting a water trap and a 2-L air flask (which had been initially back filled with dry N_2) in series in a closed-loop with the LI-6200 system and LI-6000–09 soil respiration chamber. The terracosm atmospheric CO_2 captured in the soil respiration chamber was removed using Soda Lime scrubber as a by-path in the collection system. After filling the flask with effluxed CO_2, the flask was moved to a portable extraction line and the CO_2 was extracted cryogenically (Buchmann *et al.* 1997). Back at the University of Utah, N_2O, which had been frozen out along with CO_2 in the field vacuum line, was removed using a gas chromatograph 3-m Poroplot Q column before CO_2 samples were analysed on an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Breman, Germany) operated in the dual inlet mode (Ehleringer 1991). The precision and reliability of this sampling scheme was tested using two chamberless controls (outdoor plots containing the same plants, soils, and litter) and three soil lysimeters containing the same soils but without litter and without plants.

For each terracosm, a pooled sample of newly produced needles from six seedlings was collected and frozen immediately on the same day that soil efflux CO_2 samples were collected. A pooled sample of litter from four positions surrounding the soil collar in each terracosm was collected and sealed in 10-mL screw-cap vials and secured with parafilm. Similarly, a pooled sample of top soil from the A Horizon (0–5 cm below the bottom of the litter layer) was collected from four positions around the soil collars in each terracosm. The needle samples were first dried at 70 °C for 48 h and then ground to pass through a 20-mesh sieve. For the litter and soil samples, water was cryogenically extracted in a vacuum line. Following this, the dried litter and soil samples were individually ground to pass through a 20-mesh sieve for carbon isotope ratio analyses. Soil samples were first cleaned of obvious plant fractions and then treated with 1N HCl to remove any carbonate.

The carbon isotope ratio of organic materials (needles, litter, roots, soil) was determined using an elemental analyser in conjunction with the Delta S isotope ratio mass spectrometer. Results are expressed in delta notation

($\delta^{13}C$) in ‰ (parts per thousand) relative to the Pee Dee Belemnite (PDB) standard:

$$\delta\ (‰) = (R_{sam}/R_{std} - 1) * 1000, \quad (1)$$

where R_{sam} and R_{std} are $^{13}C/^{12}C$ for the sample and standard, respectively. The external precision was ± 0.11‰ based on repeated measurements of a lab working standard (Utah cabbage).

The oxygen isotope ratios ($\delta^{18}O$) of litter and soil water were determined with the CO_2-H_2O equilibrium method modified by Socki *et al.* (1992). All oxygen isotope ratios (soil CO_2 efflux and water samples) are expressed similarly in δ units relative to the SMOW standard. External precision for $\delta^{18}O$ measurements was ± 0.23‰, based on repeated measurement of a lab working standard (Salt Lake City ground water).

Partitioning of the soil CO_2 efflux

Partitioning of the soil CO_2 efflux into components was made using a 2-endmember linear model for interpreting the $\delta^{18}O$ value of CO_2 efflux and a 3-endmember triangular model for the $\delta^{13}C$ value of the efflux. Before partitioning the soil CO_2 efflux into three components, calculations were made of the expected carbon and oxygen isotope ratios of different carbon sources within the soil profile. The $\delta^{13}C$ value of CO_2 released from rhizosphere respiration was expected to be the same as that of carbon in newly grown roots and root exudates, since there is no carbon isotopic fractionation during heterotrophic respiration (Lin & Ehleringer 1997). However, it was impractical to sample representative newly grown roots and root exudates in the terracosms for carbon isotopic analysis each time, so we measured the carbon isotope ratios of the newly produced needles as an approximate estimation (they should come from the same carbon source, i.e. the newly synthesized photosynthate). In April and October 1994, we analysed the $\delta^{13}C$ values for the fine roots (< 1 mm) from soil coring and found that they were < 0.3‰ more positive than those for the newly produced leaves in the ambient CO_2 chambers and < 1‰ more positive in the elevated CO_2 chambers (Lin *et al.* unpubl. data).

As mentioned earlier, tank CO_2 with much lower $\delta^{13}C$ values than atmospheric CO_2 was used to maintain CO_2 concentration in all terracosms, so the new carbon in the newly grown leaves and newly produced roots as well as root exudates should have had much lower $\delta^{13}C$ values than the 'old' carbon in the litter and the soil organic matter that started the terracosms. There is little carbon isotopic fractionation during the early decay of fallen plant materials (Balesdent *et al.* 1993), so we can assume that the CO_2 released from litter decomposition had a similar carbon isotope ratio to that of litter. Because we conducted this study in the second year of the treatments, there was little new litter formation and decomposition in our terracosms. Thus the carbon isotope ratio of CO_2 from litter decomposition should have been similar to that of the bulk litter in each terracosm.

Soil organic carbon consists of several fractions with different densities and turn-over times, and usually organic matter with a lower density decomposes faster than that with higher density. Thus, the $\delta^{13}C$ of CO_2 from SOM oxidation will depend mainly on the carbon isotope ratio of low-density carbon in the soil. Bird *et al.* (1996) observed that the $\delta^{13}C$ value varied among different soil size fractions, but the maximum difference for forest soils was less than 0.5‰ in most cases. Variation in SOM $\delta^{13}C$ can be attributed to differential discrimination of biochemical pathways in plants (lignin is relatively depleted in ^{13}C), but also substrate-dependent discrimination during microbial mineralization of organic matter (Mary *et al.* 1992). Since there was no direct method for measuring carbon isotope ratios of the CO_2 from SOM decomposition, we thus assumed that the $\delta^{13}C$ of CO_2 from SOM decomposition was similar to that of bulk soil organic carbon (SOC) in the surface layer. We tested this assumption by comparing the $\delta^{13}C$ value of SOC in the surface layer with that of soil effluxed CO_2 in the three soil lysimeters (the same soils but without seedlings and litter) and found that the difference between the two was 0.2–0.3‰ at most.

We assumed that the $\delta^{18}O$ value of CO_2 released from decomposition of litter was in equilibrium with the litter water, because of both the high surface-to-volume ratio in litter and the ubiquity of carbonic anhydrase in soil microbial organisms. We assumed that CO_2 efflux originating from decomposition of litter was not subject to a significant oxygen diffusion fractionation, since CO_2 from litter decomposition would be turbulently transferred from this surface layer to the atmosphere. Thus, the $\delta^{18}O$ value of CO_2 from litter decomposition was calculated from the $\delta^{18}O$ value of the litter water according to the model of Bootinga & Craig (1969):

$$\delta^{18}O_{CO2} = \alpha\ \delta^{18}O_{water} + (\alpha - 1) * 1000, \quad (2)$$

where

$$\alpha = (5.112–0.214\ t + 0.00041t^2 + 1000) * 0.00104075 \quad (3)$$

and t is the water temperature in °C.

We assumed that CO_2 evolved from rhizosphere respiration and SOM decomposition (no matter at what layers they were produced) will reach isotopic equilibrium with soil water in the top 0–5 cm layer (Ciais *et al.* 1997; Tans 1998). Before escaping into the atmosphere, the CO_2 from these two processes should have the $\delta^{18}O$ values that can be estimated from the $\delta^{18}O$ of soil water in the top layer using (2), (3). In addition, an 8.8‰

diffusion fractionation against the heavier CO_2 ($^{13}C^{18}O^{16}O$) occurs as these CO_2 efflux components diffused through to the uppermost soil layer to the litter layer (Hesterberg & Siegenthaler 1991). Thus, CO_2 efflux derived from either rhizosphere respiration or SOM decomposition should be 8.8‰ more negative in $\delta^{18}O$ value than that expected from the $\delta^{18}O$ of soil water in the top layer.

Given these assumptions, we could calculate the relative contributions of rhizosphere respiration, litter decomposition and SOM oxidation to the overall soil CO_2 efflux rate. This could be algebraically partitioned and solved by calculating the relative contribution factors [m for root carbon, n for litter carbon, and (1–m–n) for SOM carbon] from the measured isotopic compositions of the overall soil CO_2 effluxed from the soil surface as

$$\delta^{13}C_{R\text{-soil}} = m*\delta^{13}C_{R\text{-root}} + n*\delta^{13}C_{R\text{-litter}} + (1\text{-}m\text{-}n)*\delta^{13}C_{R\text{-SOM}} \quad (4)$$

$$\delta^{18}O_{R-soil} = n*\delta^{18}O_{R-litter} + (1-n)*\delta^{18}O_{R-topsoil}, \quad (5)$$

where the subscripts for $\delta^{13}C_R$ indicate the carbon source of CO_2, i.e. R-soil for total soil CO_2 efflux, R-root for root-derived CO_2, R-litter for litter-derived CO_2, and R-SOM for SOM-derived CO_2, and subscripts for $\delta^{18}O_R$ indicate the oxygen origin of soil-respired CO_2, i.e. R-litter for litter layer water and R-topsoil for the upper soil layer water.

Statistical analyses

The effects of elevated CO_2 and temperature on soil CO_2 efflux rates over the four sampling dates were tested by a two-way ANOVA. The seasonal changes in soil CO_2 efflux rate, isotopic composition of soil-respired CO_2, plant tissues and soils were tested with one-way ANOVA. The differences in the isotopic composition of effluxed soil CO_2, new needles, litter, soil organic matter, litter water, and soil water among treatments were tested using the Tukey *t*-test. All statistical analyses were performed using a PC SYSTAT 7.0 (SPSS Inc., Chicago, Illinois).

Results

Soil temperature and soil CO_2 efflux rate

The soil temperature at the top layer of the mineral soil showed a strong seasonal pattern, increasing from April to June and then decreasing after August (all $P < 0.001$, Fig. 2a). There were no significant differences in soil temperature between the ambient and elevated CO_2 treatments at either temperature treatment. However, there were consistent differences in soil temperature between ambient and elevated temperature treatments (both $P < 0.001$). The absolute difference (2.1–2.6 °C) in soil temperature between the two temperature treatments was slightly lower than the difference in air temperature between treatments (3.7 °C) (see also Tingey *et al.* 1996).

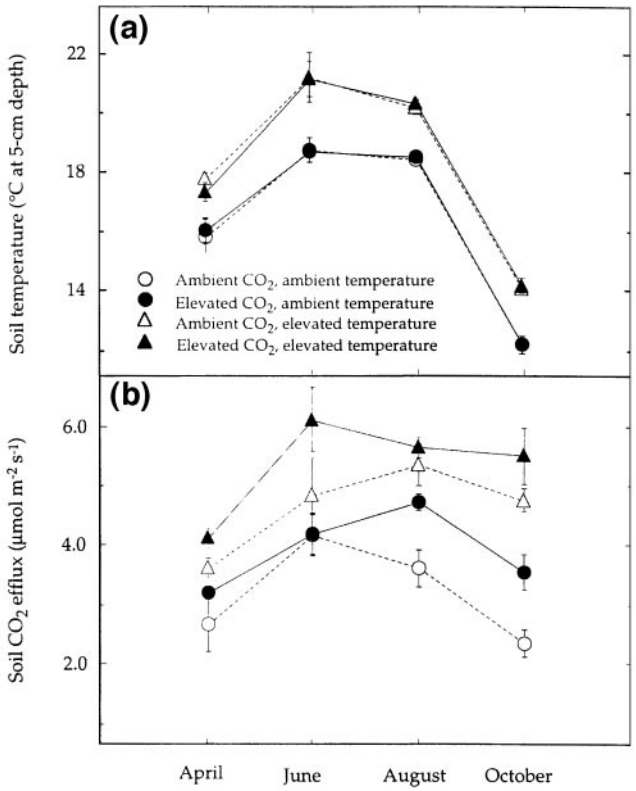

Fig. 2 (a) Soil temperature at ≈ 5-cm depth and (b) soil CO_2 efflux rate in the terracosms under different CO_2 concentration and air temperature treatments at four sampling dates in 1994. Values are the mean and standard error of three replicates for each treatment.

At ambient CO_2 and ambient temperature, soil CO_2 efflux rates ranged from 2.4 to 4.4 μmol m^{-2} s^{-1} between April and October (Fig. 2b). There was a strong seasonal trend in soil CO_2 efflux (all $P < 0.001$). Relative to the ambient CO_2 and ambient temperature treatment, soil CO_2 efflux was significantly increased by either elevated CO_2 or by elevated temperature (Fig. 2b), averaging 15% higher under elevated CO_2 (1–30%, $P < 0.05$) and 50.3% higher under elevated temperature (16–61%, $P < 0.001$). Under the combination treatment of elevated CO_2 and elevated temperature, the response was 72.6% higher (54–149%, $P < 0.001$).

Isotopic composition of soil CO_2 efflux

Under both temperature treatments, the $\delta^{13}C$ of soil CO_2 efflux was significantly lower for the elevated CO_2 treatment than for the ambient CO_2 treatment (both

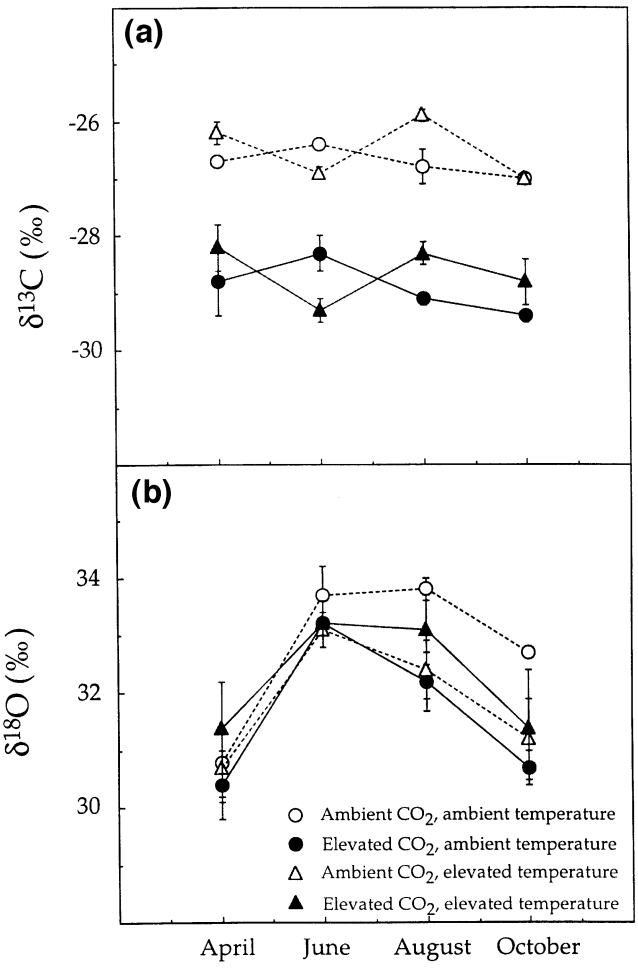

Fig. 3 Carbon and oxygen isotope ratios of soil CO_2 efflux from the terracosms under different CO_2 concentration and temperature treatments at the four sampling dates in 1994. Values are the mean and standard error of three replicates for each treatment.

$P < 0.001$, Fig. 3a). There was no significant difference in the seasonal mean $\delta^{13}C$ value between the temperature treatments at either CO_2 treatment, although the $\delta^{13}C$ differed significantly between these treatments in both June and October (both $P < 0.001$).

There was a strong seasonal trend in the $\delta^{18}O$ value of the soil CO_2 efflux in all treatments (all $P < 0.001$), with an increase between April and June, and then a decrease from June to October (Fig. 3b). There was no significant difference among the mean seasonal $\delta^{18}O$ values of the treatments. However, the $\delta^{18}O$ values of any treatment with an elevated condition (i.e, elevated CO_2 and ambient temperature, ambient CO_2 and elevated temperature, elevated CO_2 and elevated temperature) were lower than for the control (ambient CO_2 and ambient temperature) in June, August and October.

Isotopic composition of needles, litter, soil and water

The $\delta^{13}C$ values of newly grown needles ranged from – 28.4 to – 29.4‰ for the two ambient CO_2 treatments and – 34.1 to – 35.7‰ for needles in the elevated CO_2 treatments (Fig. 4a). The differences between the two CO_2 treatments were highly significant (both $P < 0.001$). However, there was no significant difference in $\delta^{13}C$ value for the newly grown needles between the two temperature treatments under either ambient CO_2 or elevated CO_2. The $\delta^{13}C$ values for the litter and the SOM were not significantly different among the four treatments (Fig. 4b,c). The $\delta^{13}C$ values for SOM were significantly higher than the values for the litter, which was also significantly higher than that for the newly grown needles in all treatments (all $P < 0.001$).

There was no significant difference in the $\delta^{18}O$ value for the litter water among treatments (Fig. 5a). However, there was a strong seasonal trend in the $\delta^{18}O$ value for the litter water in all treatments (all $P < 0.001$), with a general increase from April to August, and then a decrease from August to October (Fig. 5a). The $\delta^{18}O$ value for soil water in the top of the A Horizon was not significantly different among the four treatments, and was relatively constant throughout the four sampling dates (Fig. 5b). The $\delta^{18}O$ value for soil water was also significantly lower than the value for litter water in all treatments and at all sampling times (all $P < 0.001$).

Relative contributions of rhizosphere respiration, litter and SOM decomposition

The CO_2 originating from rhizosphere respiration, litter decomposition, and SOM oxidation had distinct carbon and oxygen isotope ratios, and the total soil CO_2 efflux had the isotope ratios within the boundaries described by the CO_2 released from these three sources (Fig. 6). Using (4) and (5), we estimated the relative contribution of these carbon sources to the overall soil CO_2 efflux for all four CO_2 and temperature treatments.

In all treatments, litter carbon was the dominant carbon source (mean of 60–64%) of the soil CO_2 efflux, followed by root carbon (23–32%), and then SOM carbon (8–18%) (Table 2). However, the relative contribution of these carbon sources varied significantly among sampling dates ($P < 0.001$ for root carbon, $P < 0.05$ for litter carbon and $P < 0.01$ for SOM carbon). At ambient CO_2, the temperature treatment had no significant effect on the relative contributions of root, litter and SOM carbon. Elevated CO_2 treatment at ambient temperature increased the contribution of root carbon in most cases, but had little effect on the relative contributions of litter and SOM carbon. Elevated CO_2 and temperature together increased the contribution of root carbon, but had no effect on the relative contribution of either litter or SOM carbon.

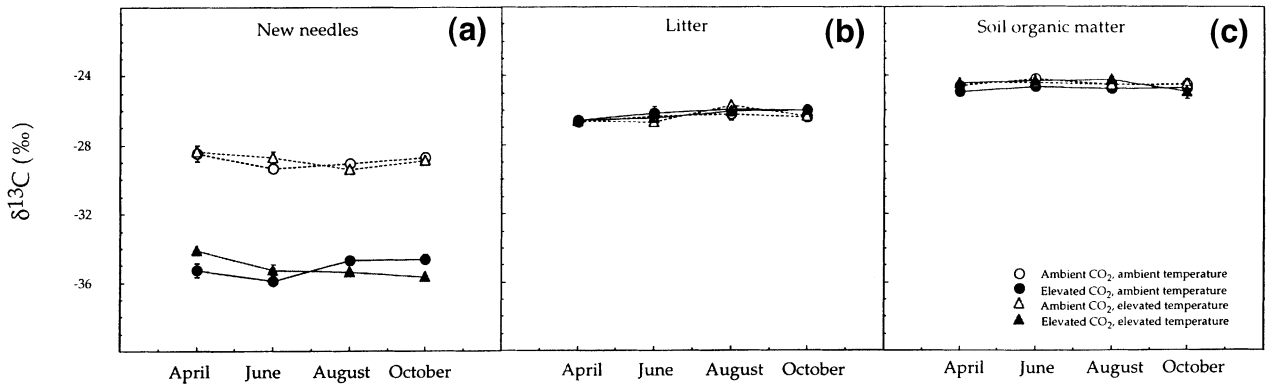

Fig. 4 Carbon isotope ratios of the (a) newly grown needles, (b) litter, and (c) soil organic matter in the terracosms under different CO_2 concentration and air temperature treatments at four sampling dates in 1994. Values are the mean and standard error of three replicates for each treatment.

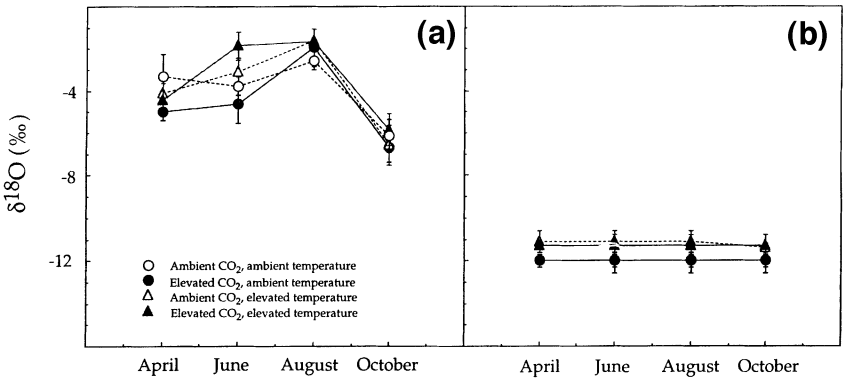

Fig. 5 Oxygen isotope ratios of the water in the (a) litter layer and (b) the top of the A horizon in the terracosms under different CO_2 concentration and air temperature treatments at four sampling dates in 1994. Values are the mean and standard error of three replicates for each treatment.

On average, elevated CO_2 treatment increased rhizosphere respiration by 79% and litter decomposition by 18%, but reduced oxidation of SOM by 14% (Table 3 and Fig. 7). Elevated temperature under ambient CO_2 increased all three components of soil CO_2 efflux, by 60%, 44% and 189% for rhizosphere respiration, litter decomposition and SOM oxidation, respectively. The elevated CO_2 and elevated temperature treatment increased rhizosphere respiration by 143%, litter decomposition by 69%, and SOM oxidation by 93% compared with the ambient-ambient treatment.

Discussion

Effect of elevated CO_2 and temperature on overall soil respiration

We observed substantial increases in the total soil CO_2 efflux by both elevated CO_2 and elevated temperature treatments. The two treatments appeared to be additive, since the increase in soil CO_2 efflux by the elevated CO_2 and elevated temperature treatment was similar to the sum of the two treatments. In this respect, our results are similar to those of many previous studies (e.g. van Veen *et al.* 1991; Körner & Arnone 1992; Johnson *et al.* 1994; Nakayama *et al.* 1994; Pajari 1995; Vose *et al.* 1995), although there are other studies showing no significant effect of elevated CO_2 on soil respiration (e.g. Oberbauer *et al.* 1986). As pointed out by Nakayama *et al.* (1994) and others, environmental conditions among the studies were quite different, so it is difficult to generalize.

Isotopic partitioning of soil respiration components

The isotope ratio of soil CO_2 efflux is influenced by both the carbon sources and by the water in the soil and litter layers. In the terracosms, the $\delta^{13}C$ of the soil carbon sources should reflect contributions from each of the three primary sources: litter, roots, and SOM. The tank CO_2 used for regulating atmospheric CO_2 concentration in all terracosms provided a much lower $\delta^{13}C$ value than typical of atmospheric CO_2, resulting in newly produced plant tissues (new needles, new roots) with distinctly more negative $\delta^{13}C$ values than the older litter and SOM carbon in the terracosms, which had been derived from field conditions. Additionally, litter in the terracosms had a lower carbon isotope ratio than SOM, which is typical (Nadelhoffer & Fry 1988; Buchmann *et al.* 1997). The

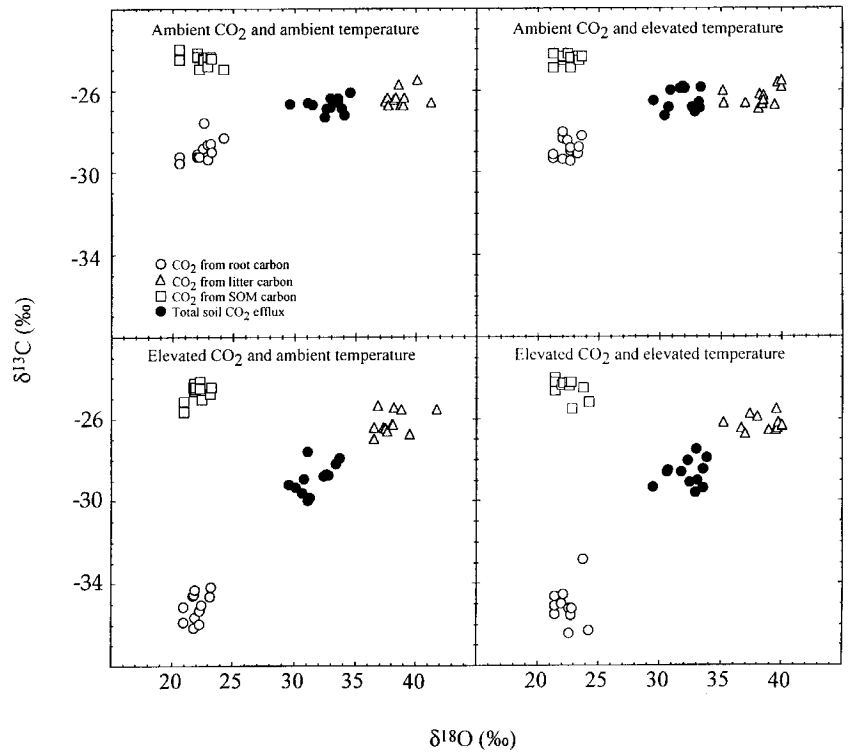

Fig. 6 The carbon and oxygen isotope ratios of total soil CO_2 efflux (closed symbols) and its three major carbon sources (open symbols) in the terracosms under different CO_2 and temperature treatments.

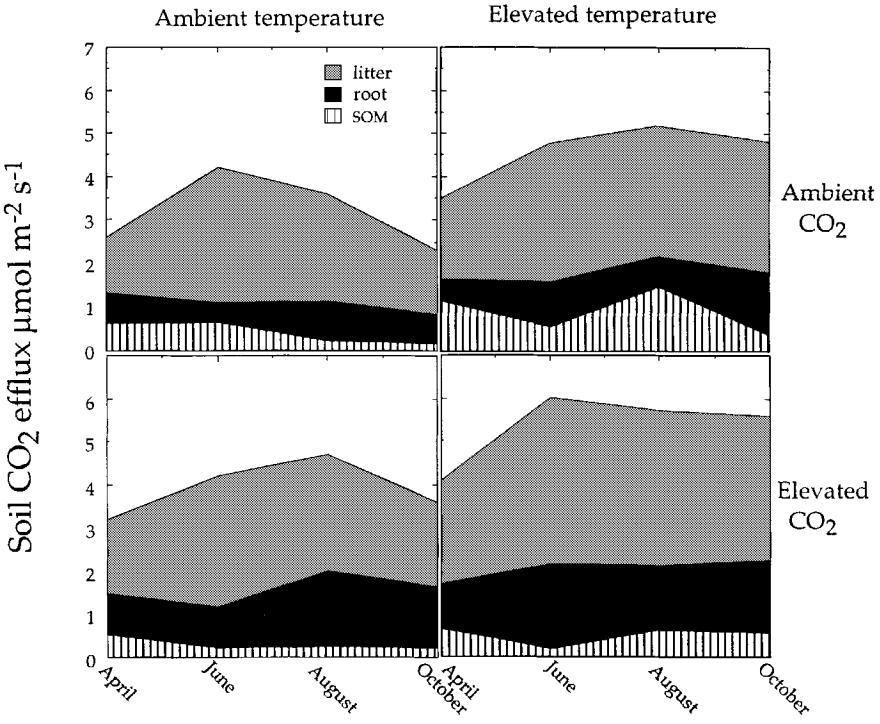

Fig. 7 Seasonal dynamics of the relative contributions of newly fixed carbon and previously formed litter and SOM carbon to total soil CO_2 efflux in terracosms under different CO_2 and temperature treatments.

combination of these three different possible $\delta^{13}C$ sources meant that using only $\delta^{13}C$ analyses (eqn 4) would not allow us to partition the relative contributions of the 'new' and 'old' carbon to the overall soil CO_2 efflux.

The water in the litter layer had a substantially higher $\delta^{18}O$ value than the water in the top of the A horizon (Fig. 5), which resulted in distinct differences in $\delta^{18}O$ values between the CO_2 derived from the litter layer (via litter decomposition) and that from the soil layer (rhizosphere respiration and SOM oxidation) (Fig. 6).

Table 2 Relative contributions (%) of root carbon (RC), litter carbon (LC) and SOM carbon (SC) to the overall soil CO_2 efflux in the terracosms under treatment combinations of ambient temperature, elevated temperature, ambient CO_2, and elevated CO_2 (mean ± SE, $n = 3$)

		Ambient CO_2		Elevated CO_2	
Sampling time	Carbon source	Ambient T	Elevated T	Ambient T	Elevated T
April-94	RC	28 ± 1	15 ± 6	29 ± 5	27 ± 7
	LC	49 ± 5	52 ± 2	55 ± 4	58 ± 1
	SC	23 ± 5	33 ± 4	16 ± 4	15 ± 2
June-94	RC	12 ± 1	22 ± 5	22 ± 4	33 ± 2
	LC	73 ± 1	67 ± 2	72 ± 4	64 ± 1
	SC	15 ± 2	11 ± 4	6 ± 1	3 ± 2
August-94	RC	25 ± 1	23 ± 3	37 ± 3	27 ± 2
	LC	69 ± 1	58 ± 3	58 ± 3	62 ± 4
	SC	6 ± 1	19 ± 1	5 ± 2	11 ± 2
October-94	RC	30 ± 2	31 ± 1	40 ± 1	31 ± 4
	LC	64 ± 1	62 ± 1	54 ± 1	59 ± 3
	SC	6 ± 2	7 ± 1	6 ± 2	10 ± 4
Average	RC	24 ± 2	23 ± 3	32 ± 4	30 ± 2
	LC	64 ± 6	60 ± 4	60 ± 4	62 ± 1
	SC	13 ± 4	18 ± 6	8 ± 3	10 ± 3

Table 3 Release rates of root, litter and SOM carbon in Douglas fir terracosms under ambient CO_2 and temperature conditions (base value) and the percentage of increase (positive) or decrease (negative) by elevated CO_2 and temperature treatments (Mean ± SE, $n = 3$)

			% change by		
Components of soil respiration	Sampling time	Base value ($\mu mol\ m^{-2}s^{-1}$)	Elevated CO_2	Elevated T	Elevated CO_2 & T
Rhizosphere respiration	Apr-94	0.75 ± 0.12	22 ± 5	19 ± 8	47 ± 14
	June-94	0.48 ± 0.10	98 ± 30	135 ± 5	319 ± 24
	Aug-94	0.91 ± 0.11	94 ± 15	– 20 ± 18	62 ± 9
	Oct-94	0.70 ± 0.05	101 ± 5	106 ± 3	142 ± 29
	Average	0.71 ± 0.09	79 ± 19	60 ± 36	143 ± 62
Litter decomposition	Apr-94	1.29 ± 0.22	37 ± 12	46 ± 8	87 ± 23
	June-94	3.07 ± 0.15	– 5 ± 9	4 ± 6	28 ± 11
	Aug-94	2.47 ± 0.19	11 ± 8	25 ± 12	43 ± 11
	Oct-94	1.50 ± 0.13	29 ± 9	100 ± 11	119 ± 23
	Average	2.08 ± 0.42	18 ± 9	44 ± 21	69 ± 20
SOM oxidation	Apr-94	0.63 ± 0.19	– 17 ± 17	88 ± 23	– 4 ± 9
	June-94	0.62 ± 0.03	– 68 ± 5	– 18 ± 20	– 64 ± 21
	Aug-94	0.23 ± 0.06	– 3 ± 2	563 ± 37	180 ± 25
	Oct-94	0.16 ± 0.06	31 ± 5	121 ± 34	258 ± 54
	Average	0.41 ± 0.12	– 14 ± 20	189 ± 128	93 ± 75

Using the two isotopic mass balance equations (eqns 4 and 5) simultaneously, we could partition the three components of soil CO_2 efflux. For all treatments and sampling dates, the largest contributor to total soil CO_2 efflux was litter decomposition, followed by the contributions of rhizosphere respiration and SOM oxidation. This dual-isotope approach also allowed us to examine the responses of these three components of soil CO_2 efflux to the CO_2 and temperature treatments (see below), and also should be applicable to field situations wherever there are substantial isotopic variations in both carbon and oxygen isotope ratios for soil CO_2 efflux.

Responses of soil CO_2 efflux components to elevated CO_2 and temperature

Release of newly formed carbon (via rhizosphere respiration) responded the most to the combined elevated CO_2 and temperature treatment. The elevated CO_2 treatment alone significantly enhanced the release of newly fixed

carbon at all sampling dates. Elevated temperature reduced the relative proportion of root carbon in April and August, but enhanced its release in June and October. Elevated CO_2 and elevated temperature together stimulated release of newly formed carbon to a greater extent throughout the year. It has been demonstrated previously that elevated CO_2 increases carbon allocation to fine roots and increases root exudation, and thus enhances soil CO_2 efflux (e.g. Norby *et al.* 1992; Johnson *et al.* 1994; Rogers *et al.* 1994).

Litter decomposition responded significantly to all treatments involving elevated ambient CO_2 or elevated temperature. We are unsure why litter decomposition was so responsive to elevated CO_2. Previous studies have shown that elevated CO_2 changes the quality of new litter, probably affecting litter decomposition (Coûteaux *et al.* 1991; van de Geijin & van Veen 1993). However, there was little new litter formation from the new needles grown under the CO_2 and temperature treatments, since our study was conducted in the second year of the treatments. Perhaps elevated CO_2 increased plant carbon fixation and allocation to roots, enhancing root exudation and turnover processes, as has been suggested by others (Berntson & Woodward 1992; Norby *et al.* 1992; Rogers *et al.* 1994). The increased carbon likely stimulated microbial communities and nutrient cycling processes, thereby increasing litter decomposition to meet their nitrogen requirements (van Veen *et al.* 1991). This usually occurs when inorganic nitrogen availability is low as is the situation in this Douglas-fir system. Soil nitrogen was low ($< 0.1\%$), and NO_3^- or NH_4^+ was never detected in soil solutions (detection limits for NO_3^- and NH_4^+ were 0.04 mg L^{-1} and 0.10 mg L^{-1}, respectively).

Although SOM carbon contributed a relatively small proportion to overall soil-respired CO_2 (Table 2), release of this previously formed carbon was also increased by elevated temperature in most cases (Table 3, Fig. 7). We recognize that the large relative increase in oxidation of SOM may be an artifact stemming from the small basal values for the ambient CO_2 and ambient temperature treatment. However, SOM is a large reservoir of global carbon, recently estimated at about 1600 Pg, which is more than twice the atmospheric CO_2-C pool (Schimel 1995). Small changes in the size of the soil organic carbon pool could significantly affect atmospheric CO_2 concentrations. The annual flux of CO_2 from soils to the atmosphere is estimated at 76.5 Pg C y^{-1}, which is 30–60% greater than terrestrial net primary productivity (Raich & Schlesinger 1992). If soil organic carbon is reduced under elevated atmospheric CO_2 or elevated temperature conditions, then soils represent a significant carbon source, increasing the amount of the carbon to the atmosphere (Jenkinson *et al.* 1991). On the other hand, elevated CO_2 usually stimulates photosynthetic carbon uptake, so the overall effects of increasing atmospheric CO_2 on carbon balance in the forest ecosystems of northwestern USA will be determined by the responses in both above-and below-ground processes.

Possible errors associated with assumptions

In this study, we had to make some assumptions for the calculations of isotopic signals for different CO_2 sources (see Materials and methods section). First, we assumed that the CO_2 from rhizosphere respiration has similar $\delta^{13}C$ value to that of newly grown needles. If the $\delta^{13}C$ of the newly grown needles was more negative than that of the active roots and root exudates, we would have underestimated the contribution of rhizosphere respiration to the overall soil CO_2 efflux (see Fig. 6), especially in the ambient CO_2 chambers where the difference between the 'new' and 'old' carbons were small (2–5‰). Our spot measurements indicate that the mean difference in the $\delta^{13}C$ between the newly grown needles and the fine roots (< 1 mm) was < 0.3‰ for the ambient CO_2 chambers and < 1.0‰ for the elevated CO_2 chambers (Lin *et al.* unpubl. data). Thus, the possible errors associated with this assumption may cause about 5–15% (0.3 vs. 2–5‰ for the ambient CO_2 chambers and 0.9 vs. 6–12‰ for the elevated CO_2 chambers) deviation from the estimated values for the relative contributions, but will not change the general patterns discussed in the above sections.

A second assumption is that the $\delta^{13}C$ of CO_2 from SOM matter is similar to that of bulk SOC. As mentioned earlier, bulk SOC is made of several fractions which may decompose at different rates and have different isotopic compositions (Bird *et al.* 1997). Therefore, the actual $\delta^{13}C$ of CO_2 from SOM oxidation will be different from that of bulk SOC. If the carbon contributed to the soil CO_2 efflux is lighter (more negative in $\delta^{13}C$) than bulk SOC, we may then underestimate the relative contribution from SOM oxidation to the overall soil CO_2 efflux (see Fig. 6), especially in the ambient CO_2 chambers because of the relatively small difference in $\delta^{13}C$ between SOC and the CO_2 from total soil respiration. Fortunately, the difference $\delta^{13}C$ among SOC fractions was often less than 0.5‰ in forest ecosystems (Bird *et al.* 1996), so the error associated with this assumption will be marginal and again should not alter the patterns described in the previous sections. Our test with the soil lysimeters also indicated that this was probably the case in our Douglas fir seedling systems (Lin *et al.* unpubl. data). It is clear that further characterization of soil isotopic compositions are needed to more accurately estimate the contribution of SOM oxidation.

In addition, the partitioning of the soil CO_2 efflux into its components depends in part on the extent to which

CO_2 diffusing through a soil layer to the atmosphere does or does not come into isotopic equilibrium with the soil water in a particular layer (Tans 1998). This matter is not well understood at the moment and there are few experimental data available. While Hesterberg & Siegenthaler (1991) assume full expression of the diffusion isotope fractionation factor for $^{12}C^{18}O^{16}O$ between the soils and the atmosphere (8.8‰) as did Farquhar *et al.* (1993) for the gradient between leaves and the atmosphere, Ciais *et al.* (1997) calculated that a value of 3.29‰ was required in order to balance biosphere-atmosphere fluxes at the global level. Given our assumption of turbulent transfer from the litter layer and diffusive transfer from the soil to the atmosphere, the effective overall expression of the diffusion isotope fractionation factor may be closer to that predicted by Ciais *et al.* (1997). However, experimental studies are required to determine the extent to which CO_2 fluxing out of soils is in equilibrium with particular soil and litter layers. From the data presented in Fig. 6, it is clear that an exact interpretation of the soil CO_2 efflux data will require a better understanding of the diffusion and equilibrium isotope fractionation factors.

It is worth mentioning that the difference in $\delta^{13}C$ between the 'new' carbon (i.e. root carbon) and 'old' carbon (including both litter and SOC) in the ambient CO_2 treatments was quite small (2–5‰) in relation to natural variations within each carbon pool among replicate chambers (Fig. 6). Thus, the partitioning results based on the ^{13}C analysis alone would result in significant errors (e.g. at the ambient CO_2 treatments we would have seen little contributions from rhizosphere respiration to total soil CO_2 efflux). However, there was a large difference in $\delta^{18}O$ between litter water and soil water, but there was no difference in $\delta^{18}O$ between the CO_2 from SOM oxidation and the CO_2 from rhizosphere respiration (Fig. 6). Therefore, the partitioning based on the ^{18}O analysis alone would only allow separation of soil respiration into litter component and the other component contributed from rhizosphere respiration and SOM oxidation. It is the combination of the ^{13}C and ^{18}O analysis that made it possible to partition all three components of soil respiration (Fig. 6). Our trial application of this dual-isotope technique to the Douglas fir terracosms under different CO_2 and temperature conditions (Fig. 7, Tables 2,3) suggests that this novel method can provide new insights into the responses of carbon metabolism in forest ecosystems to future climate changes.

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